Tetrahedron, 1966, Vol. 22, pp. 3173 to 3179. Pergamon Press Ltd. Printed in Northern Ireland

CHEMICAL STUDIES ON HELIANGINE

A NEW SESQUITERPENE LACTONE ISOLATED FROM THE LEAVES OF *HELIANTHUS TUBEROSUS* L

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(*Received* 10 *January* 1966; *in revised form* 10 *March* 1966)

Abstract—The structure of heliangine has been found to be I on the basis of its chemical studies.

HELIANGINE was isolated from *Helianthus tuberosus* L. as a plant growth regulator was found to have an inhibitory effect on *Avena* curvature or straight growth test.[1,2] In the present work, the chemical structure of heliangine has been investigated. Heliangine is the first germacrane-type sesquiterpene having plant physiological activities, although some sesquiterpenes such as xanthinin,[3] helminthosporol[4] and abscisin II[5] are also active substances.

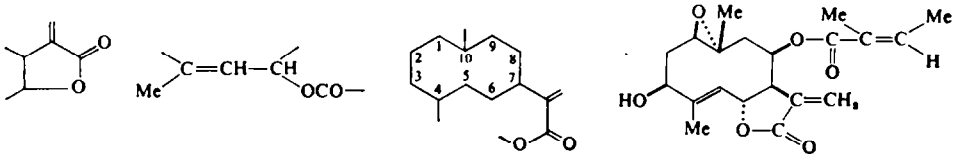
Heliangine (I), $C_{20}H_{26}O_6$, m.p. 227–229°, $[\alpha]_D^{25}$ −110°, contains a secondary hydroxyl group as shown by its IR band at 3450 cm^{-1} and by the formation of a monoacetate. In the IR spectrum, bands of α,β-unsaturated-γ-lactone at 1754 cm^{-1}, ester of an α,β-unsaturated acid at 1715 cm^{-1} and double bonds at 1660 cm^{-1} are observed. The C-methyl value determined by Kuhn-Roth method was 3·64, and in the NMR spectrum four methyl groups are found, one of which as a singlet at τ 8·5 and three methyl signals linked to the double bonds near τ 8·2. Since formaldehyde was liberated by ozonolysis of I, existence of one exocyclic methylene group was assumed. By the hydrogenation of I over palladized carbon, a mixture of dihydroheliangine (II) and tetrahydroheliangine (III) was obtained. The maximum UV absorption of I at 207·5 mμ (ε:23,800) is no longer recognized in II and the end absorption extensity of II at 207·5 mμ decreases to 12,800. In the IR spectrum of II, the band at 1754 cm^{-1} of I shifts to 1770 cm^{-1}, corresponds to a γ-lactone. The lactonic function of heliangine can therefore be defined as a partial formula (A). This is confirmed from the NMR spectrum, namely two proton signals at τ 3·7 (doublet J = 2 c/s) and τ 4·3 (doublet J = 2 c/s) in I disappeared and one new C-methyl signal appeared instead at τ 8·9 (doublet J = 7 c/s) in II.

Compound I was hydrolysed with barium hydroxide in aqueous alcohol to give tiglic acid and a diol, $C_{15}H_{20}O_5$, named helianginol (IX). The newly appeared hydroxyl group is also secondary as shown by the NMR spectrum and by the formation of dihydrohelianginol diacetate. The NMR spectrum of I indicated that the *trans* isomer may be present in the natural material, for the chemical shift corresponding to the β-proton of the acid moiety in I (τ 3·09) is more likely to that of methyl tiglate

[1] H. Shibaoka, *Plant and Cell Physiol.* **2**, 175 (1961).
[2] M. Mitsuhashi and H. Shibaoka, *Plant and Cell Physiol.* **6**, 87 (1965).
[3] P. G. Deuel and T. A. Geissman, *J. Amer. Chem. Soc.* **79**, 3778 (1957).
[4] S. Tamura, A. Sakurai, K. Kainuma and M. Takai, *Agr. Biol. Chem.* **27**, 738 (1963).
[5] K. Ohkuma, F. T. Addicott, O. E. Smith and W. E. Thiessen, *Tetrahedron Letters* 2529 (1965).

(τ 3·27) than that of methyl angelate (τ 4·02). The hydrogenation of I over platinum oxide proceeded in two stages. The mild hydrogenation gives III, which was hydrolysed to α-methylbutyric acid and dihydrohelianginol (X). The latter was also obtained by hydrolysis of II and agreed with the reduction product of IX.

The more vigorous hydrogenation afforded deoxyhexahydroheliangine (V) and a mixture of two isomeric hexahydroheliangine (IVa, b) having no more ethylenic group. A pair of two coupled proton signals at τ 3·5 (doublet J = 11 c/s) and τ 4·7 (doublet J = 11 s/c) of III disappeared in either IVa or IVb, and only one proton signal



A B C D

remained at τ 4·85 (multiplet). At the same time, one methyl signal linked to an ethylenic group at τ 8·1 shifts to near τ 8·8–8·9. These facts indicate the existence of partial formula (B).

When III, where only one double bond remained, was oxidized with chromic acid in acetic acid, tetrahydroheliangine (VI) was obtained, which contained an α,β-unsaturated ketone characterized by the IR band at 1697 cm^{-1} and has the maximum UV absorption at 246 mμ (ε: 5,950). On the other hand, IVa was also oxidized in the same manner as III giving hexahydroheliangine (VIII), but the product contained an isolated ketone, confirmed by IR band at 1694 cm^{-1}. Further one methylene group of VIII must be adjacent to the carbonyl group because of the positive Zimmermann test.

Now we have clarified the characters of the five oxygens and their neighboring structure. The last oxygen must form an ethereal ring because it is neither hydroxyl nor carbonyl. By treating with sulfuric acid, VI is isomerized to the dienone compound (VII) as shown by its IR bands at 1650 and 3460 cm^{-1} and the maximum UV absorption at 249·8 mμ (ε: 9,430). The hydroxyl group should be derived from the sixth oxygen. It is tertiary one because it cannot be acetylated with acetic anhydride–pyridine, and its proton signal of —O*H* in the NMR spectrum in dried DMSO is surely singlet. This means that one terminal of the ether should be linked to the same carbon with the methyl group appearing always near τ 8·4–8·6 (singlet). In the NMR spectrum of VII two ethylenic proton signals appear at τ 3·72 (doublet J = 17·2 c/s) and τ 3·11 (doublet J = 17·2 c/s). It means that another terminal of the ethereal ring links to the carbon of new ethylenic group, namely α or β position to the ketone in VI. If the former is correct, the marked proton signal (O═C—C*H*—O) must be observed clearly in the NMR spectrum, and the positive Zimmermann test cannot be explained from the structure. Considering the result of the dehydrogenation, it can be concluded that the ethereal ring is an epoxide group.

Two azulenic compounds were obtained by dehydrogenation of II over palladized carbon, and one of them was a blue oil (XII) having UV and visible region absorption spectrum similar to that of chamazulene, and the other was a violet oil (XIII). From

the partial structure and the two azulenic compounds, it is reasonable to explain that helianginol (IX) contains a germacrane lactone (C).

Now, we are left then with three constitutions I, XIV and XV for heliangine. The formula XIV is unacceptable because the partial formula (B) is still maintained after the hydrolysis of II and acetylation of X. This is confirmed by comparing the behavior between C-6 and C-8 proton signals in the NMR spectra. The signal of C-6 proton (τ 4·47), coupled with that of C-5 proton, doesn't shift in X and XI. On the other hand the signal of C-8 proton (τ 4·87) shifts to τ 5·72 in X (pyridine). It appears again τ 4·8 by acetylation. Although there is no experimental evidence to decide

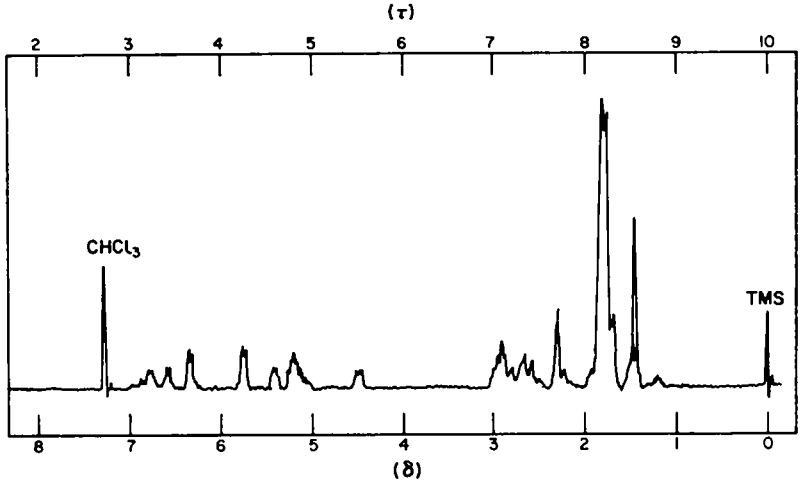


FIG. 1. NMR Spectrum of Heliangine in $CDCl_3$

whether it is I or XV, preference for formula I is based on the fact that no germacrane-type compounds oxidized as XV have ever been discovered in natural products. The stereostructure of heliangine has been confirmed to be (D) or its mirror image by X-ray crystallography of dihydroheliangine chloroacetate by Nishikawa *et al.*[6]

EXPERIMENTAL[7]

Isolation of Heliangine (I)

The leaves of *H. tuberosus* growing at Fukuchiyama experimentation farm were dried at 60° for 6–7 hr. The dried leaves (21·5 kg) were macerated in 180 l. MeOH and after 10 days at room temp filtered off, and the filtrate concentrated *in vacuo* to ca. 4 l. The green viscous residue was dissolved in a mixture of 5 l water, 5 l. MeOH and 7 l. ligroin. The separated water–MeOH layer was washed with ligroin and the MeOH evaporated. The aqueous solution was extracted 3 times with 1 l. $CHCl_3$ and the $CHCl_3$ solution concentrated to 200 ml and poured on a column (45 × 5 cm) of 1300 ml silica gel (Wako gel Q-23). The first eluate of $CHCl_3$ (1 l.) was discarded and the second eluate of $CHCl_3$–MeOH (95:5; 3 l.) was evaporated, and the residue rechromatographed through a column

[6] M. Nishikawa, K. Kamiya, A. Takabatake, H. Oshio, Y. Tomiie and I. Nitta, *Tetrahedron* in press.

[7] The m.ps were taken in open glass capillaries and are uncorrected. The UV or visible region spectra were measured in a Hitachi Recording Spectrophotometer EPS-2. The IR spectra were determined on KBr disks in a Perkin-Elmer 21 or Hitachi EPI-II. The NMR spectra were measured in $CDCl_3$ solution, unless indicated otherwise, in a Varian A-60. The mass spectra were measured in a Hitachi RMU-6D. The rotation measurement were carried out in a Perkin–Elmer 141. Silica gel used for column or TLC was Silica gel G (Merck), unless otherwise stated.

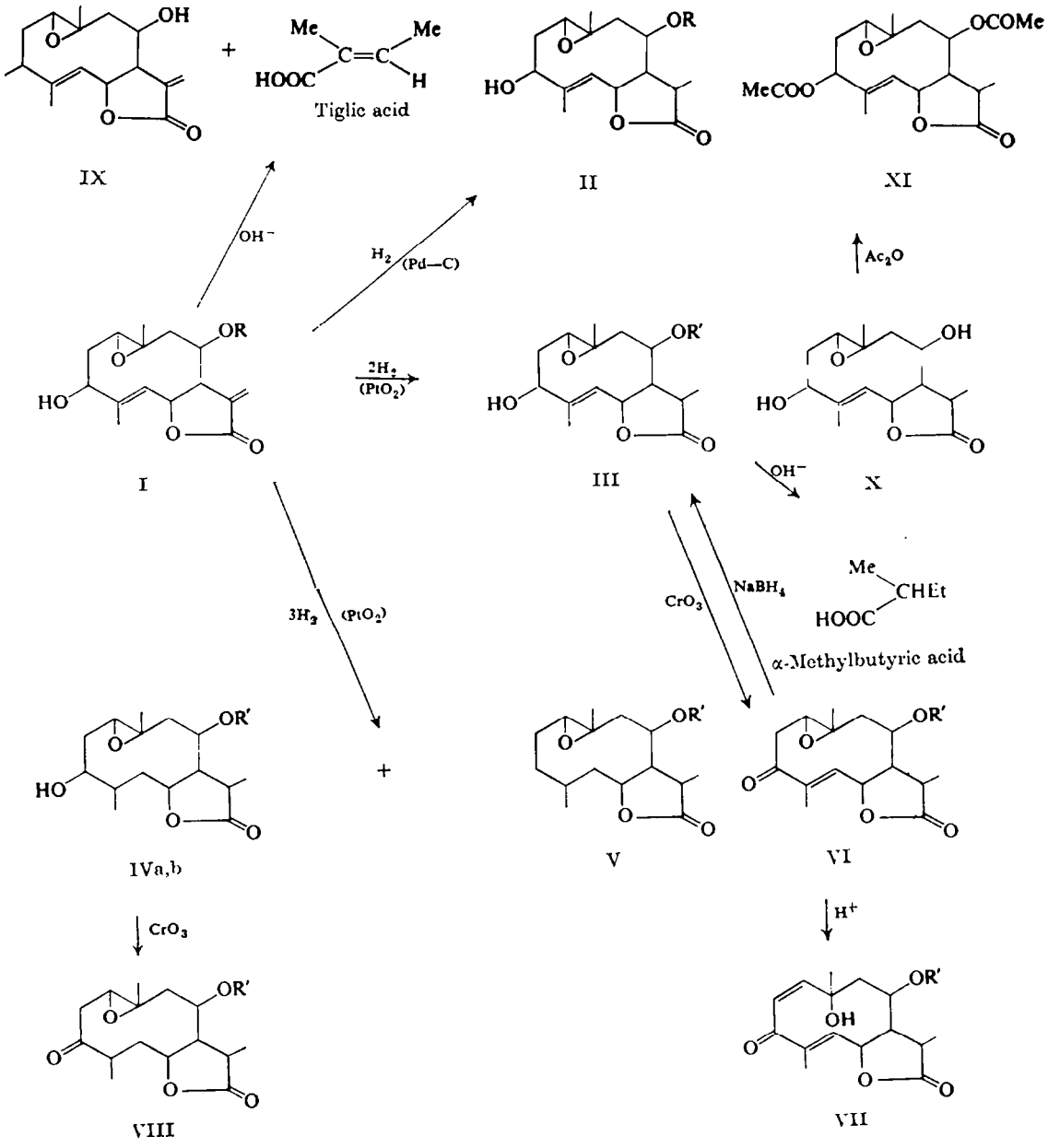


FIG. 2. (Continued to another Fig. 2)

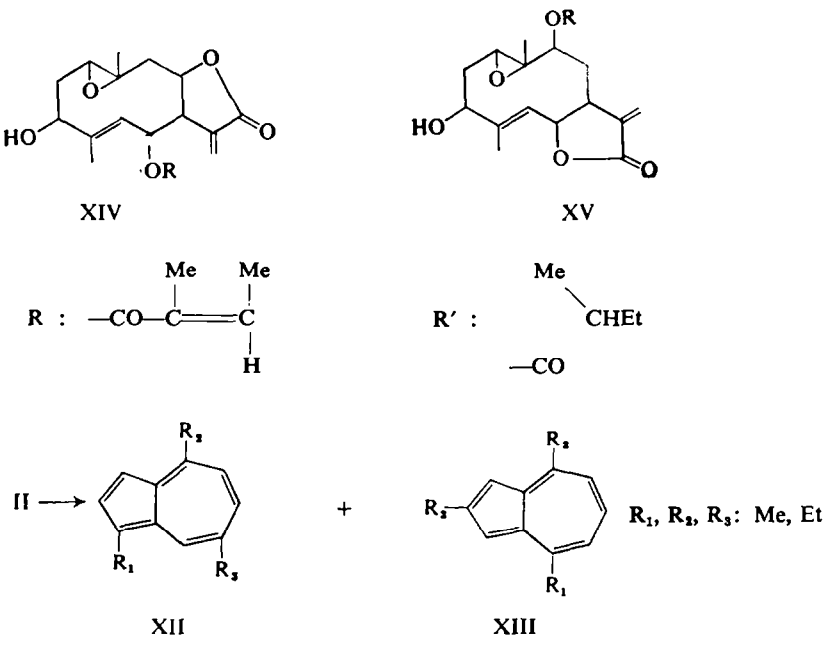


FIG. 2. (Continued from another Fig. 2)

(45 × 3 cm) of alumina (300 ml, Merck standardisiert) with $CHCl_3$. By concentration of the effluent, crude crystals of heliangine (I) were obtained in 0·05% yield. These were recrystallized from MeOH giving colorless prisms, m.p. 227–229°. (Found: C, 66·33; H, 7·15; mol. wt. 362 (MS), 391 (osmotic method). $C_{20}H_{26}O_6$ requires: C, 66·28; H, 7·23%; mol.wt. 362·41%.) $[\alpha]_D^{25}$ −110° (*c*, 0·5, $CHCl_3$). λ_{max}^{EtOH} 207·5 mμ (ε: 23,800). ν_{max} 3450, 1754, 1715, 1660 cm^{-1}.

I-*acetate*. Compound I (489 mg) was treated with Ac_2O and AcONa at 95° for 2 hr. After cooling, the reaction mixture was poured into ice water and the resulting white precipitate recrystallized from MeOH to give colorless prisms, m.p. 182–186° (320 mg, 58·6%). (Found: C, 65·28; H, 6·84; mol. wt. 404 (MS). $C_{22}H_{28}O_7$ requires: C, 65·33; H, 6·98%; mol. wt. 404·22.) ν_{max} 1737 cm^{-1} (CH_3CO).

Ozonolysis of heliangine

A solution of I (300 mg) in $CHCl_3$ (100 ml) was ozonized at below 0° for 30 min, and the pale colored mixture immediately steam distilled. The distillate was collected in a solution of dimedone in EtOH and the organic solvent removed by redistillation. On cooling the aqueous solution, 40 mg of a formaldehydedimedone complex, m.p. 187·5°, was obtained (16·5%). No depression was observed in a m.m.p. with an authentic sample.

Dihydroheliangine (II)

A solution of I (724 mg) in AcOH (40 ml) was hydrogenated over 10% Pd-C (1 g) and 1·5 moles H_2 absorbed in 25 min. After filtration, the organic solvent was evaporated *in vacuo* and from the colorless oily residue, crystals were separated by addition of a small amount of MeOH. Recrystallization from MeOH yielded 412 mg of colorless plates, m.p. 205–206° (56·6%). (Found: C, 65·91; H, 7·59. $C_{20}H_{28}O_6$ requires: C, 65·91; H, 7·74%.) ν_{max} 1770 cm^{-1} (γ-lactone). τ 8·90 (doublet J = 7 c/s) (HC–CH_3). The mother liquor was concentrated *in vacuo* and acetylated as for I giving 110 mg of tetrahydroheliangine acetate (13·5%).

II-*acetate*. Compound II (88 mg) was acetylated as for I giving 80 mg of colorless needles, m.p. 204–206° (81·6%). (Found: C, 64·95; H, 7·42. $C_{22}H_{30}O_7$ requires: C, 65·01; H, 7·44%.)

II-*chloroacetate*. Compound II (200 mg) and chloroacetylchloride (2 ml) was treated with DMF (5 ml) for 5 hr, and the reaction mixture poured into ice water (150 ml). The precipitate was filtered

off and recrystallized from MeOH to give 90 mg of colorless plates. m.p. 156–159° (37%). (Found: C, 60·34; H, 6·75; Cl, 8·67; mol. wt. 440 (MS). $C_{22}H_{29}O_7Cl$ requires: C, 59·92; H, 6·61; Cl, 8·04%; mol. wt. 440·89.)

Tetrahydroheliangine (III)

A solution of I (724 mg) in AcOH (50 ml) was hydrogenated over PtO_2 (200 mg) and 1·97 moles H_2 absorbed. The resulting colorless gum was chromatographed through silica gel, and from the eluate of C_6H_6–AcOEt (1:1), 652 mg of a powder was obtained (89·1%). (Found: C, 65·25; H, 8·25. $C_{20}H_{30}O_6$ requires: C, 65·55; H, 8·25%.) ν_{max} 1770 (γ-lactone), 1736 cm^{-1} (ester).

III-*acetate*. Compound III (361 mg) was acetylated to give 184 mg of colorless bars, m.p. 194–195° (45·8%). (Found: C, 64·58; H, 7·74. $C_{22}H_{32}O_7$ requires: C, 64·68; H, 7·90%.)

Hexahydroheliangine (IVa, b) *and deoxyhexahydroheliangine* (V)

A solution of I (724 mg) in AcOH (40 ml) was hydrogenated over PtO_2 (50 mg) for 80 min and 3·1 moles H_2 absorbed. The colorless gumming product was chromatographed through silica gel, and by elution with C_6H_6–AcOEt (1:1), V and two isomers (IVa, b) of hexahydroheliangine were separated. Compound V was recrystallized from C_6H_6–petr. ether giving 24 mg of colorless crystals, m.p. 157° (3·3%). (Found: C, 68·15; H, 8·99. $C_{20}H_{32}O_5$ requires: C, 68·15; H, 9·15%.) No hydroxy band was observed in the IR spectrum. Compound IVa was recrystallized from C_6H_6 to give 294 mg of colorless leaflets, m.p. 151–154° (39·9%). (Found: C, 65·27; H, 8·83. $C_{20}H_{32}O_6$ requires: C, 65·19; H, 8·75%.) $[\alpha]_D^{25}$ −47·7° (*c*, 1·0, EtOH). IVb was reprecipitated from C_6H_6 to afford 290 mg of a white powder, m.p. 157° (39·4%). (Found: C, 65·06; H, 8·69. $C_{20}H_{32}O_6$ requires: C, 65·19; H, 8·75%.) $[\alpha]_D^{23}$ −71·4° (*c*, 1·0, EtOH).

IVa-*acetate*. Compound IVa (100 mg) was acetylated as for I to give 72 mg of colorless needles, m.p. 164–165° (64·6%). (Found: C. 64·60; H, 8·18. $C_{24}H_{34}O_7$ requires: C, 64·37; H, 8·35%.) $[\alpha]_D^{23}$ −28·4° (*c*, 1·0, EtOH).

IVb-*acetate*. Compound IVb (100 mg) was acetylated to yield 65 mg of colorless fine crystals, m.p. 147–148° (58·3%). (Found: C, 64·33; H, 8·18. $C_{24}H_{34}O_7$ requires: C, 64·37; H, 8·35%.) $[\alpha]_D^{23}$ −60·2° (*c*, 1·0, EtOH).

Hydrolysis of heliangine

To a solution of I (724 mg) in MeOH (40 ml), a solution of $Ba(OH)_2$ (324 mg) in water (40 ml) was added and the mixture refluxed for 2 hr. The yellow mixture was then concentrated *in vacuo* to ca. 20 ml and the residue was extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed through silica gel, and elution with C_6H_6–AcOEt (1:1) gave colorless crystals of IX, which were recrystallized from MeOH to yield colorless needles, m.p. 191–192° (21 mg or 3·8%). (Found: C, 64·32; H, 7·01; mol. wt. 280 (MS). $C_{15}H_{20}O_5$ requires: C, 64·27; H, 7·19%; mol. wt. 280·31.) ν_{max} 3450, 3500 cm^{-1} (2-OH). The aqueous solution was acidified with HCl, extracted with CH_2Cl_2, the extract concentrated and the residue sublimated at 70° under 20 mm Hg press to give 16 mg of colorless crystals, m.p. 61° (30·5%). (Found: C, 59·67; H, 7·88. $C_5H_8O_2$ requires : C, 59·98; H, 8·05%.) λ_{max}^{EtOH} 218 mμ (ε: 8,740). The product was identical with authentic tiglic acid in the IR spectrum.

Hydrolysis of dihydroheliangine

Compound II gave X (50%) and tiglic acid by hydrolysis as for I. Recrystallized from acetone, X had m.p. 202–203°. (Found: C, 64·16; H, 7·80. $C_{15}H_{22}O_5$ requires: C, 63·81; H, 7·85%.)

X-*diacetate* (XI). Compound X (250 mg) was dissolved in a mixture of Ac_2O (3 ml) and pyridine (1 ml) and heated at 90° for 3 hr. The product was purified by recrystallization from C_6H_6–petr. ether to yield colorless needles, m.p. 209–211° (18·5%). (Found: C, 62·34; H, 7·11. $C_{19}H_{26}O_7$ requires: C, 62·28; H, 7·15%.) ν_{max} 1740 cm^{-1} ($2CH_3CO$).

Hydrolysis of tetrahydroheliangine

Hydrolysis of III yielded X (21·8%) and α-methylbutyric acid. The latter was identified with authentic D,L-α-methylbutyric acid from its IR spectrum.

Tetrahydroheliangінone (VI)

A solution of III (5 g) in AcOH (100 ml) was mixed with a suspension of CrO_3 (3 g) in AcOH (50 ml) and maintained at 10° overnight. The reaction mixture was poured into ice water and the resulting colorless fine crystals recrystallized from C_6H_6, m.p. 174–175° (2·5 g, 50·3%). (Found: C, 65·46; H, 7·64. $C_{20}H_{28}O_6$ requires: C, 65·91; H, 7·74%.) λ_{max}^{EtOH} 246 mμ (ε: 5,950). ν_{max} 1697 cm^{-1} (ketone).

Reduction of tetrahydroheliangінone to tetrahydroheliangine

To a solution of VI (130 mg) in tetrahydrofuran (3 ml) $NaBH_4$ (100 mg) was added in small portions and left for 3 days. The reaction mixture was chromatographed through silica gel and eluted with AcOEt. The fractions showing the same R_f-value on TLC were combined and concentrated. The resulting colorless solid was immediately acetylated as for I, and the colorless bars (from MeOH) found to be identical with III m.p. 191°, in IR spectrum and m.m.p. (192–194°).

Isomerization of tetrahydroheliangінone

Compound VI (320 mg) was stirred for 30 min in a mixture of EtOH (20 ml) and 1% H_2SO_4 (10 ml) at room temp. After neutralization, the organic solvent was evaporated *in vacuo* and the resulting precipitate recrystallized from C_6H_6 to give colorless platelets of VII, m.p. 176–178° (25·0%). (Found: C, 65·91; H, 7·97. $C_{20}H_{28}O_6$ requires: C, 65·91; H, 7·74%.) λ_{max}^{EtOH} 249·8 mμ (ε: 9,430). ν_{max} 1650 (ketone), 3460 cm^{-1} (OH).

Hexahydroheliangінone (VIII)

Compound IVa (450 mg) was oxidized as for III to give colorless crystals, which were recrystallized from C_6H_6–petr. ether, yielding 209 mg of colorless prisms, m.p. 146–148° (46·7%). (Found: C, 65·49; H, 7·97. $C_{20}H_{30}O_6$ requires: C, 65·55; H, 8·25%.) ν_{max} 1694 cm^{-1} (ketone).

Dehydrogenation of dihydroheliangine over palladized carbon

A mixture of II (2 g) and 10% Pd-C (2 g) was heated in an atmosphere of N_2 at 310–350° for 30 min. The distilled yellow green oil was dissolved in a mixture of water–petr. ether and the blue colored petr. ether layer extracted with 80% H_3PO_4. After washing with C_6H_6, the H_3PO_4 solution was diluted with water and reextracted with petr. ether. The extract was chromatographed through alumina (Merck standardisiert) and eluted with petr. ether to give two azulenic compounds. One of them was a blue oil (XII) and its UV and Vs spectra resembled those of chamazulene.[8] The other was a violet oil (XIII) and seemed to be a 2,4,8-trisubstituted azulene.[9]

Acknowledgements—We thank Prof. Dr. Yamaki *et al.* (Tokyo University) for permission of this investigation and bioassay, and Dr. Tanaka, division chief of our laboratories, for his kind advice. Thanks are also due to members of Fukuchiyama experimentation farm of our company for their cultivation of the material plant, to Mr. Kawashima for perparation of the crude extract and to members of our analytical section for their analyses and determination of physical constants.

[8] K. Takeda, T. Kubota and W. Nagata, *Chem. Pharm. Bull. Tokyo* **1**, 241 (1953). We thank Dr. Nagata for a copy of original chart.

[9] M. Gordon, *Chem. Revs.* **50**, 127 (1952).